

DIRECTIONS FOR PERFORMING THE DAVIS TEST* FOR URINARY ALA

The column chromatography procedure which you are about to perform for the determination of urinary delta-aminolevulinic acid (ALA) as an indicator for early lead poisoning is divided into three sequential phases:

- PHASE 1 Removal of porphobilinogen and urea from the urine which would interfere with the analysis of ALA.
- PHASE 2 Column elution of ALA.
- PHASE 3 Colorimetric determination of eluted ALA.

Components of the Davis ALA Test Kit for 40 determinations of urinary ALA.

(Any of the three numbered catalog items listed below may be ordered separately....)

1. Catalog No. 95502, Support Rack with drain tray and numbered pipet guide (non-disposable).

2. Catalog No. 95004, disposable Piggy-back Column Units with following reagents and standard:

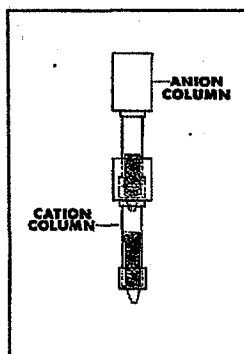
- a. Bottle containing 500 ml of 1.0 M sodium acetate solution* (68 gm of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ diluted with water to 500 ml). Refrigerate upon delivery.
- b. Bottle containing 20 ml of acetylacetone (2,4-pentanedione).
- c. Bottle containing 10 gm (pre-weighed) of reagent-grade p-dimethylaminobenzaldehyde.
- d. Bottle containing 10 ml of stock solution of ALA hydrochloride standard dissolved in acetate buffer, pH 4.6.** Refrigerate upon delivery. Each ml of stock solution contains 125 μg of ALA.HCl which is equivalent to 100 μg of pure ALA.

3. Catalog No. 95501, one pound bottle of 72% perchloric acid (quantity sufficient for 200 tests).

NOTE: 420 ml of glacial acetic acid is required but is not supplied with the kit because of postal shipping regulations.

BEGIN TEST PROCEDURE

STEP 1



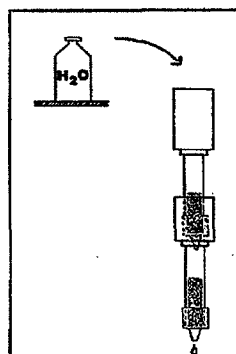
TECHNIQUE

Remove numbered pipet guide and place piggy-back column units in support rack.

NOTE:

The piggy-back column units arrive with the top and bottom resins in the correct position. This two column set is referred to as the column unit. Each urine sample requires one column unit for the determination of ALA.

STEP 2



TECHNIQUE

Apply 10 ml of distilled water to the column unit and allow to drain through both columns into drain tray.

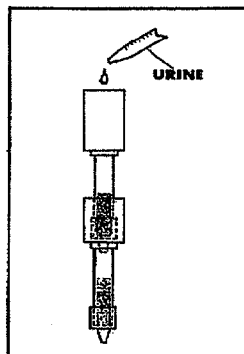
SUGGESTION

Use of a 10 ml Cornwall continuous, syringe-type pipetter will permit fast, automatic delivery of the desired quantity of water to each column unit.

*Davis, J.R. and Andelman, S.L., Arch. Environ. Health, 15:53-59 (1967)

**0.8% boric acid present as a preservative.

STEP 3



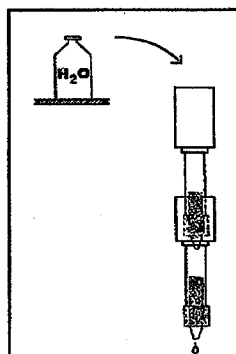
TECHNIQUE

Replace numbered pipet guide. Apply 0.5ml of random urine sample to the top of the column unit by inserting pipet through the correctly numbered hole in pipet guide.

SUGGESTION

Disposable pipets have been found to be quite economical when testing large numbers of urine samples. Allowing the pipets to remain in the numbered holes until all of the samples have been added has been found to eliminate the possibility of applying two urine samples to the same column unit.

STEP 4



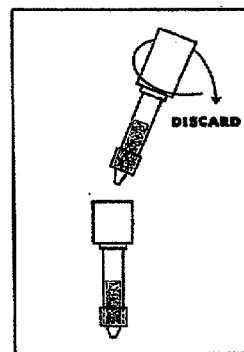
TECHNIQUE

Remove pipets from column units. Remove numbered pipet guide. Apply 10ml of distilled water to each column unit and allow to drain through columns into drain tray. Repeat two more times for a total water wash of 30ml.

SUGGESTION

It is important that all column units are completely drained of the previous water wash before applying the next aliquot of 10ml of water to the column unit. This will prevent column overflowing.

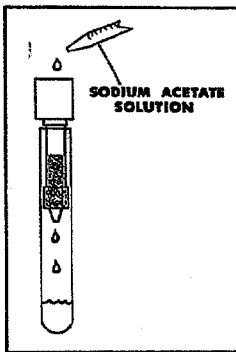
STEP 5



TECHNIQUE

Remove and discard top disposable column from piggy-back column unit. The 6 aliquots of ALA hydrochloride stock solution can now be pipetted into test tubes as working standards as indicated in the section on Preparation of a Standard Calibration Curve as shown on page 3. Subsequent steps for both the standards and unknowns are identical beginning with the addition of 1.0M sodium acetate.

STEP 6



TECHNIQUE

Remove bottom columns from support rack and place in empty numbered 20x150mm Pyrex test tubes held in a standard test tube rack. Add 7.0ml of 1.0M sodium acetate to each column and collect the drained liquid in the test tube.

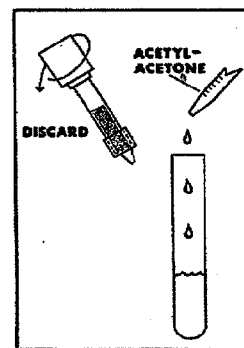
SUGGESTION

20x150mm disposable borosilicate glass culture tubes have been found to be quite economical when testing large numbers of urine samples.

Note:

It is important that the test tubes be of the suggested size in order to hold the column at the correct height. It is also of importance that all column units be fully drained in order to completely elute the ALA.

STEP 7



TECHNIQUE

Remove and discard bottom disposable column from test tube. Add 0.2ml of acetylacetone to the contents of the test tube and mix.

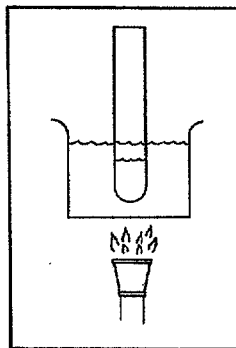
SUGGESTION

Use of a 1 ml Repipet® will permit the rapid and precise delivery of acetylacetone. Use of a Vortex mixer will permit rapid and easy mixing of test tube contents.

Note:

Amounts of acetylacetone greatly in excess of 0.2 ml will inhibit final color development.

STEP 8



TECHNIQUE

Place test tube rack containing test tubes in a boiling water bath (90°C or greater) for 10 min., after which the rack should be removed from water bath and test tubes allowed to cool to room temperature.

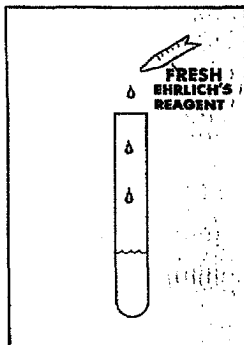
SUGGESTION

In addition to the standard temperature controlled water baths commonly available, a Bunsen burner or an electric hot plate can be used. Time for cooling of test tubes can be markedly decreased by immersing test tube rack in a pan of room temperature water.

Note:

Allowing test tubes to remain in boiling water bath greatly in excess of 10 minutes will inhibit final color development.

STEP 9



TECHNIQUE

Add 7.0ml of fresh Ehrlich's Reagent to test tube and mix. Allow to stand for 15 min. for completion of color development.

SUGGESTION

Use of a 10ml Repipet® or an automatic 10ml syringe pipet will permit the rapid and easy delivery of fresh Ehrlich's Reagent. Use of a Vortex mixer will permit the rapid and easy mixing of test tube contents.

STEP 10

TECHNIQUE

Read optical density of final reaction mixture at 553 mμ in a standard laboratory colorimeter.

SUGGESTION

Normal values of urinary ALA produce a faint yellow to faint pink color while abnormally elevated values of urinary ALA produce a distinct red color. It is therefore possible in a large screening program to immediately eliminate all non-red colors by visual inspection. A very accurate quantitation of the mg% ALA in the red samples can then be obtained by plotting the optical density of the red samples against known concentrations of pure ALA standard carried through the identical procedure.

INSTRUCTIONS FOR PREPARING FRESH EHRlich's REAGENT

1. Add 420 ml of glacial acetic acid to an empty, amber stoppered bottle.
2. Add entire 10 gm of reagent-grade p-dimethylaminobenzaldehyde (DMAB) to the glacial acetic acid and mix. If all 40 determinations of ALA are to be performed on the same day, then add 80 ml of 72% perchloric acid to the entire acetic acid solution of DMAB. This final solution is fresh Ehrlich's Reagent and will remain stable up to 6 hours.
3. Note: If all 40 determinations are not to be performed on the same day, then add 19 ml of 72% perchloric acid to every 100 ml aliquot of acetic acid solution containing the added DMAB in order to prepare the desired quantity of fresh Ehrlich's Reagent. The remainder of the acetic acid solution of DMAB not containing perchloric acid will remain stable for several months if kept in a refrigerator where it will solidify. Subsequent thawing on the day of the analysis is performed by running warm water over the outside of the bottle following which perchloric acid can then be added as outlined above to prepare fresh Ehrlich's Reagent.

PREPARATION OF A STANDARD CALIBRATION CURVE

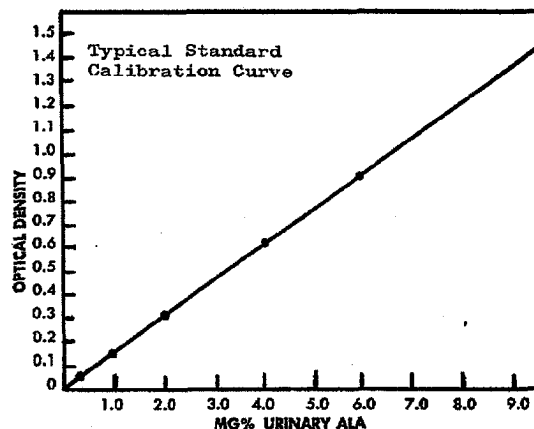
1. Six aliquots of the ALA hydrochloride stock solution (0.01 ml, 0.05 ml, 0.10 ml, 0.20 ml, 0.30 ml and 0.50 ml) are used as working standards.*

Vol. of Standard Stock Solution (1 ml = 125 μg of ALA.HCl)	μg of ALA per total reaction volume	Corresponding mg% of urinary ALA for a 0.5 ml sample of urine applied to the column
0.01 ml	1 μg	0.20 mg%
0.05 ml	5 μg	1.00 mg%
0.10 ml	10 μg	2.00 mg%
0.20 ml	20 μg	4.00 mg%
0.30 ml	30 μg	6.00 mg%
0.50 ml	50 μg	10.00 mg%

2. Dilute each of the six working standards to 7.0 ml mark with 1.0 M sodium acetate using graduated test tubes.
3. Pipet 7.0 ml of 1.0 M sodium acetate into an empty test tube to be used as a reagent blank.
4. Add 0.2 ml of acetylacetone to each tube and mix.
5. Place tubes in a boiling water bath for 10 minutes, remove and cool to room temperature.
6. Add 7.0 ml of fresh Ehrlich's Reagent to each tube, mix and let stand for 15 minutes for complete color development.

*Note: 125 μg ALA.HCl corresponds to 100 μg ALA

7. Set a colorimeter to 100% transmission at 553 mμ using the reagent blank.
8. Read the optical density of the standards and unknowns.
9. Prepare a standard curve by plotting the concentrations of the standards against optical density.
10. Read the concentration of urinary ALA in the unknowns (0.5 ml urine sample applied to the column unit) in terms of mg% from the standard calibration curve obtained.



INTERPRETATION OF URINARY ALA VALUES		
Urinary ALA Range (mg%)	Urinary ALA Code	Relationship to Lead Exposure
0.00 - 0.54	Normal	None
0.55 - 0.99	Trace	None
1.00 - 1.49	1 plus	Moderate
1.50 - 1.99	2 plus	Heavy
2.00 - 2.99	3 plus	Severe
3.00 - 5.99	4 plus	Critical
6.00 -10.00	5 plus	Overwhelming

EXPECTED RESULTS FOR THE DAVIS TEST FOR URINARY ALA	
Limit of detectability in urine...	0.03mg%
Sensitivity of test.....	0.15 µg
Variation between duplicate samples ±	2%
Recovery of ALA from column.....	97%

ORDERING INFORMATION

Catalog No.	Description	Pricing
TEST KITS		
95002	20 Piggy-back Column Units (Complete with reagents* and standard)	\$ 34.50
95004	40 Piggy-back Column Units (Complete with reagents* and standard)	60.00
95020	200 Piggy-back Column Units (Complete with reagents* and standard)	207.00
95501	* Note: Perchloric acid (72%) is not supplied with the kit but is available in one pound bottles sufficient for 200 tests.	4.00
SUPPORT RACK FOR HOLDING DISPOSABLE COLUMNS DURING TEST PROCEDURES		
95502	Support rack for 40 Column Units with drain tray and numbered pipet guide.	13.75
REPIPETS® FOR EXTRA CONVENIENCE		
95801	1 ml, with 950 ml amber bottle	47.50
95802	10 ml, with 950 ml amber bottle	47.50
All prices are FOB shipping point, either Richmond, California, or New York City. Terms: Net 30 days.		



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